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Understanding fungal wood decay:
Determining the role of oxalic acid in the fungal ECM

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Background:

Wood decay fungi produce a ß-glucan extracellular matrix (**ECM**) sheath that surrounds the fungal hyphae and connects these hyphae to their substrate – wood cell walls. These fungi also produce oxalate as part of that EMC, and our **Goal** was to determine what role oxalate may play relative to the ß-glucan fungal ECM to better understand the mechanisms involved in fungal wood degradation in nature.

Objectives:

1. Explore how oxalate impacts the formation of the ECM, and whether it may cause chemical or structural changes in the ECM.
2. Understand how oxalate may bind to ß-glucan and if so, whether oxalate may still diffuse (in-part) into the wood cell wall to impact fungal decay chemistry in the digestion of the wood cell wall by fungi.

Methods:

Three analyses were conducted:

A. Oxalate Diffusion from ß-Glucan Gels
Aqueous ß-glucan gels ranging from 0%-6% concentration were made with a single concentration of oxalic acid (100 mM). After the gels set, diffusion of oxalate from these gels over time was measured using ion exclusion HPLC.

B. Yield Rheometry of Gels With, and Without, Oxalate Present
"Yield rheometry" allows the "strength" or mechanical properties of a gel to be measured. Here, we measured a 7% ß-glucan gel made with or without oxalate, and then measured the yield point of the gels 24h after gel-set.

C. Raman Spectroscopy of Gels to Assess Chemical Bonding
The interaction between oxalic acid and β-glucan in gels was measured by adding nanogold to the gels to enhance Raman spectral features for identification of specific chemical bonds. The gels were then analyzed by Raman spectroscopy. Raman spectra were compared with theoretical calculations using density functional theory by colleagues at WPI.

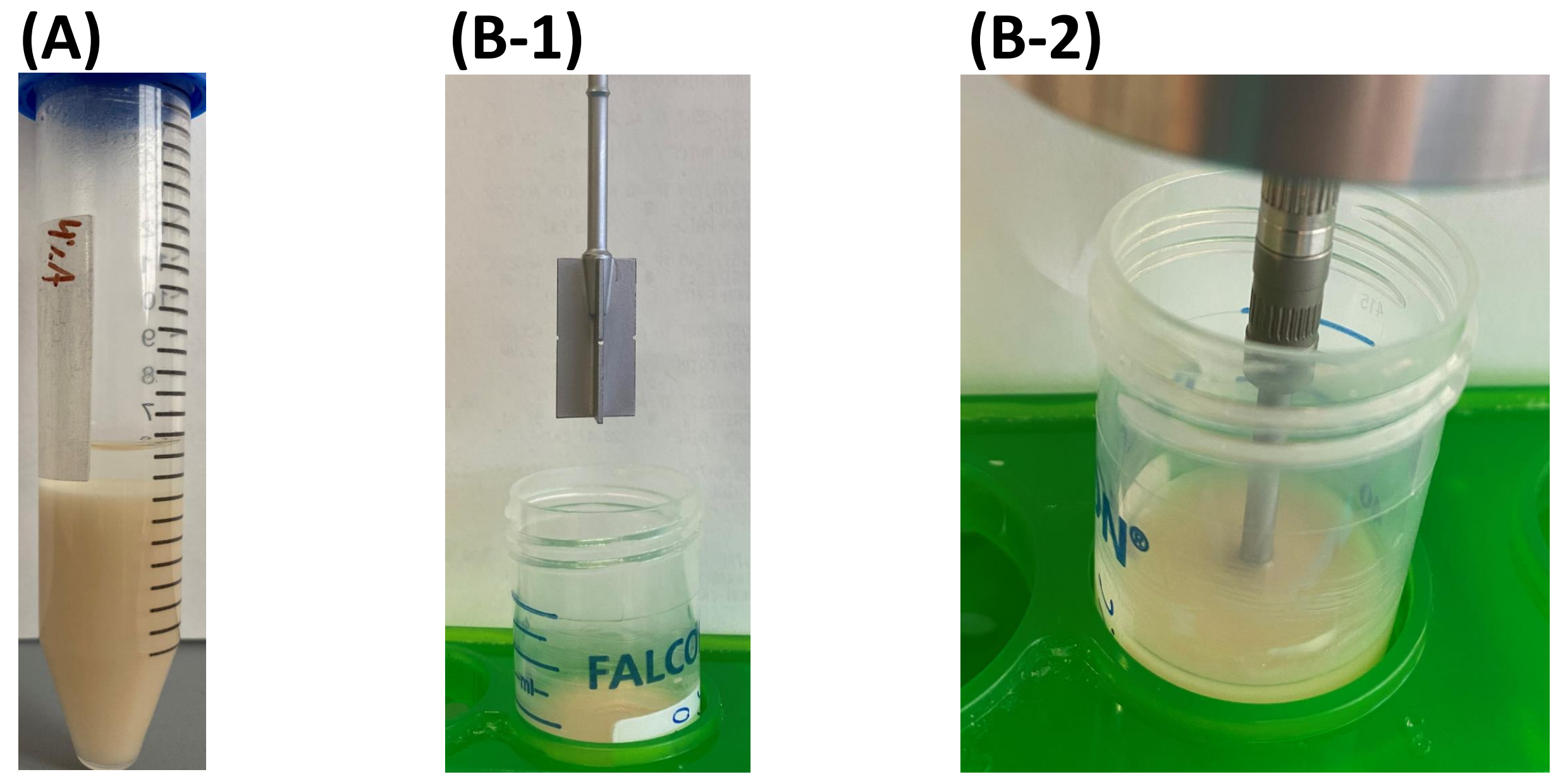

Figure 1. (A) Experimental setup of β-glucan Gels for the Oxalic acid diffusion/retention test. (B-1) Yield viscosity determination with paddle ready for placement above the gel, and (B-2) Paddle inserted into gel at the immersion mark.

Results:

Oxalic acid diffusion/retention

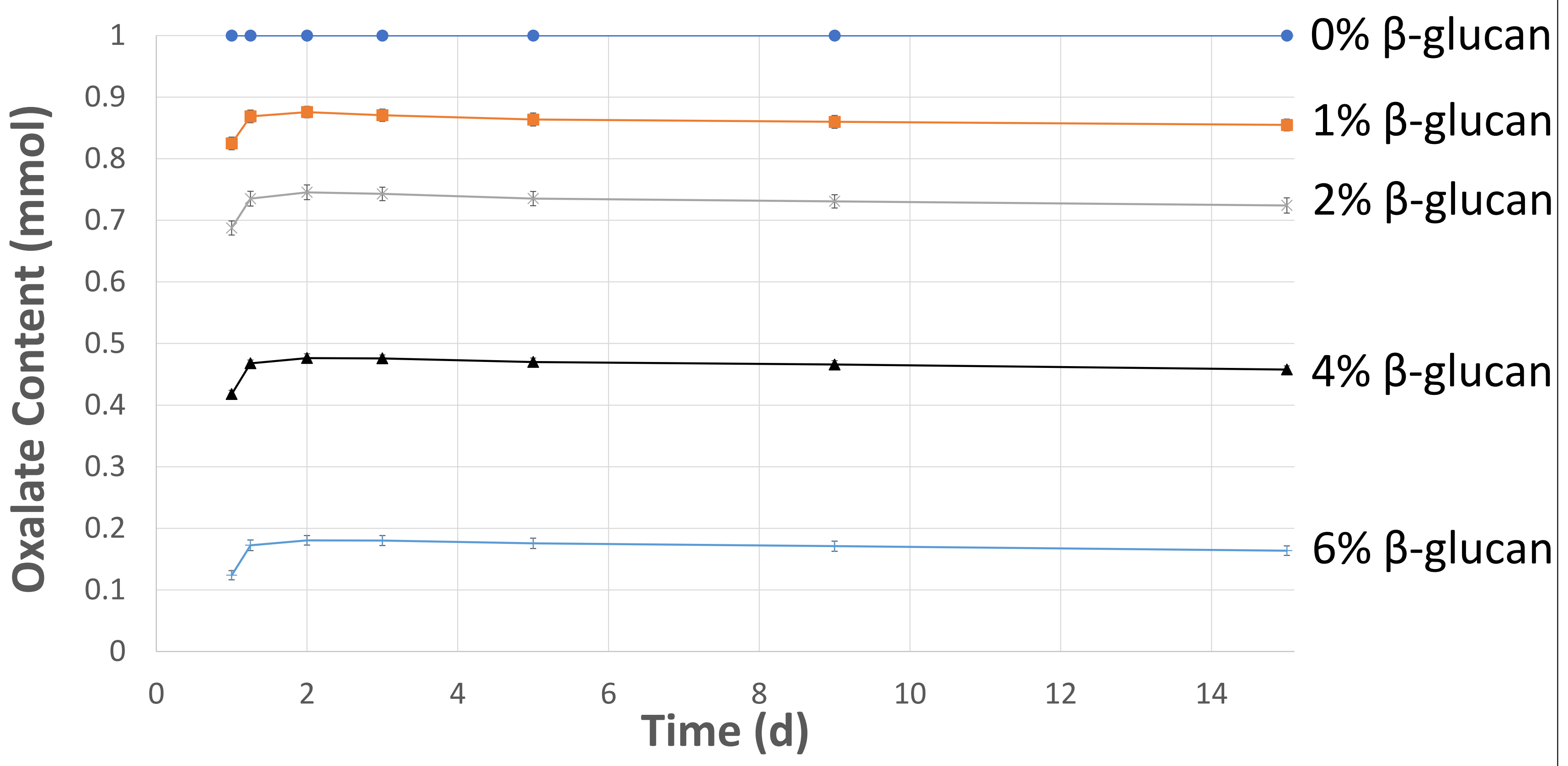

Figure 2. Diffusion of oxalate from ß-glucan gels; each initially containing 1 mmol oxalic acid. Curves represent the total oxalic acid that diffused over time into water that was layered above the gels.

Findings: As the concentration of ß-glucan in a synthetic oxalate-EMC gel increased, the amount of oxalate diffusing from those gels into a liquid layer decreased (Figure 2). This demonstrates that ß-glucan binds oxalate, but that a portion of the oxalate can diffuse from the ß-glucan gel, depending on the ß–glucan concentration.

Yield viscosity determination

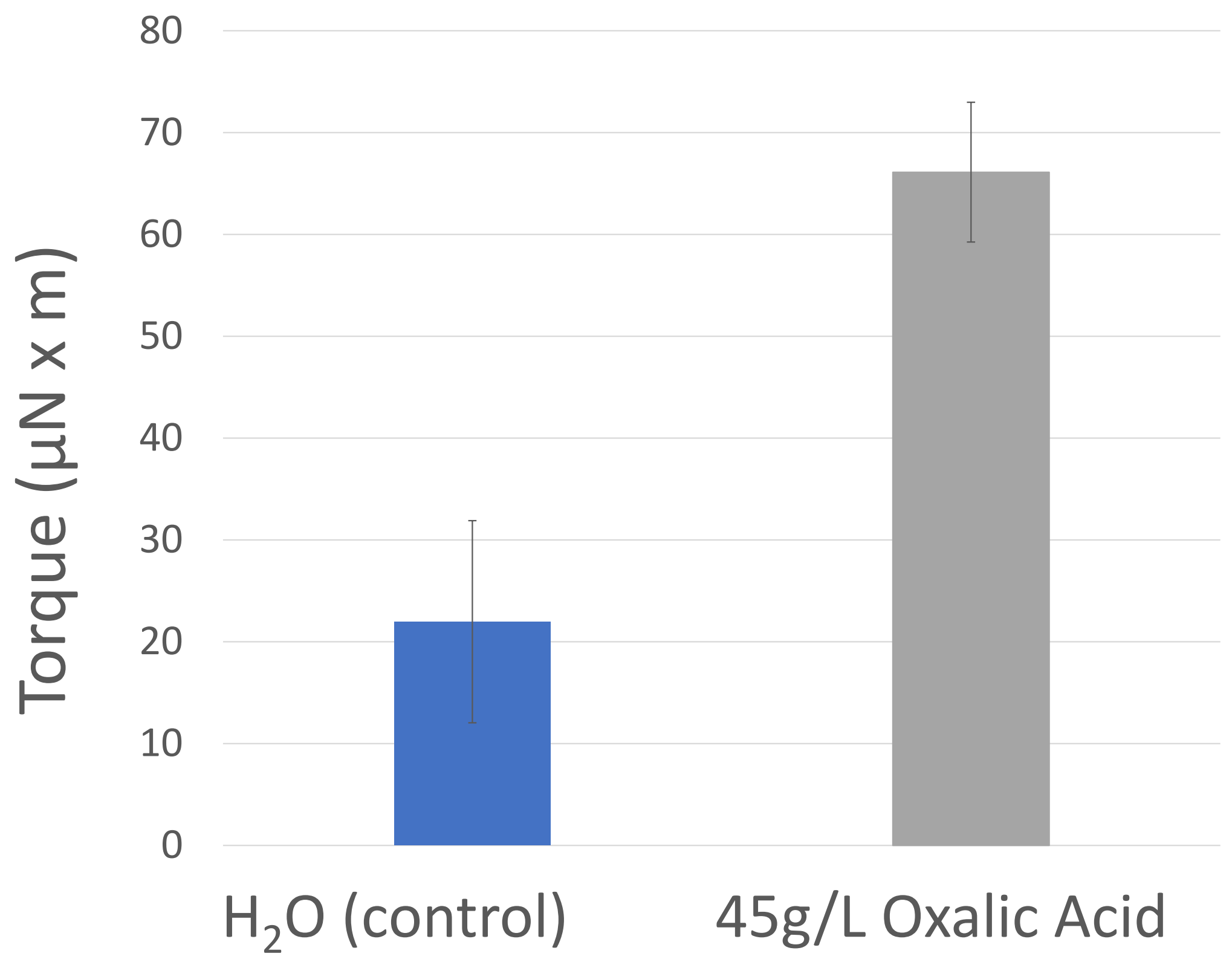

Figure 3. Yield viscosity of 7% ß-glucan gels at the gel rupture (yield) point. The difference in viscosity between gels made with and without oxalate is statistically significant.

Findings: The viscosity of ß-glucan gels increased significantly in presence of oxalic acid (Figure 3). The increased stiffness of the ß-glucan gel demonstrates that oxalic acid is causing either chemical or structural modifications of the ß-glucan matrix.

Raman spectroscopy for structural chemistry

Analysis of any ß-glucan and oxalic acid bonding which occurs is currently ongoing (Figure 4).

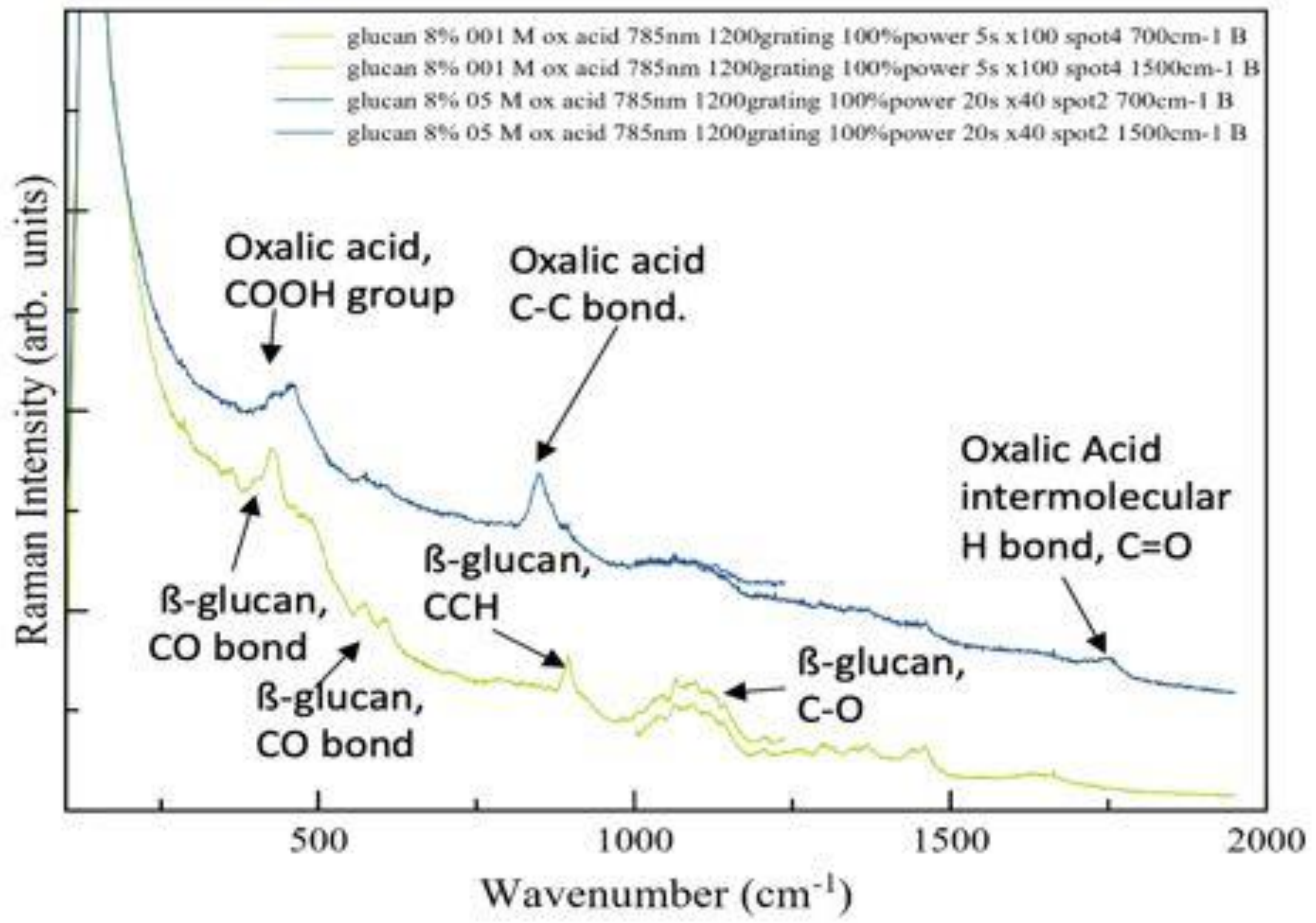

Figure 4. Raman spectra of ß-glucans Gels. Distinct bands for oxalic acid and ß-glucan, interaction between them can be usually observed as new bands or shifting of original bands.

Findings: Current data suggest intermolecular interactions may be present, but their nature is currently still being assessed.

Conclusions:

- Oxalic acid binds to ß-glucan, and it plays a role in the formation of ß-glucan gels by interacting directly with the ß-glucan matrix to increase its viscosity. This interaction may be employed by the fungi to regulate the diffusion of oxalate, and also perhaps the diffusion of other fungal metabolites. This supports the hypothesis that fungal control over metabolite diffusion promotes attack of the wood cell wall during wood decay initiation via non-enzymatic mechanisms.
- Due to the mild conditions at which gels were prepared, we hypothesize that the interaction between oxalic acid and ß-glucan has a non-covalent character. Further analysis (in collaboration with WPI colleagues) is on-going to help understand the nature of the interaction and the diffusional properties of the ß-glucan gel with oxalic acid.

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